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# Molecular Phylogenetic Studies in Rosaceae

Christopher S. Campbell

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**Final Report for Period:** 10/1998 - 09/2001  
**Principal Investigator:** Campbell, Christopher S.  
**Organization:** University of Maine  
**Title:**  
 Molecular Phylogenetic Studies in Rosaceae

**Submitted on:** 12/20/2001  
**Award ID:** 9806945

### Project Participants

#### Senior Personnel

**Name:** Campbell, Christopher  
**Worked for more than 160 Hours:** Yes  
**Contribution to Project:**

#### Post-doc

#### Graduate Student

#### Undergraduate Student

#### Research Experience for Undergraduates

### Organizational Partners

#### Other Collaborators or Contacts

(A brief summary of the impact of this proposal on the research of the first three participants is given in the section on Contributions):

1. The designated postdoctoral associate, Dr. Rodger C. Evans, worked on this grant from June 1999 through August 2000, when he took a tenure-track position at Acadia University in Wolfville, Nova Scotia.
2. Dr. Timothy A. Dickinson took a full-year sabbatical leave (August 1999 to June 2000) with us to learn molecular systematic techniques for his work on the genus *Crataegus* (Rosaceae).
3. Jenny Smedmark, a Ph.D. student from Sotckholm University spent most of the month of July 2000 at the University of Maine collecting DNA
4. T. Eriksson, collaborator on Rosoideae, visited UMaine twice during the period of this grant. We discussed work on the Rosoideae and the overall phylogeny of the family.
5. Three undergraduate students have been involved in this project. Shane Sims, a Biology major, worked in the lab during the summer of 1999. Margaret Cox is a junior majoring in Clinical Laboratory Sciences, but she has a strong interest in plants. She has worked on various aspects of this project, although most of her time has been devoted to other projects. Matthew Arsenault, a junior Biology major, is currently working in the lab. He has been trained in all phases of lab work, from PCR to cloning to sequence analysis (He will do DNA extractions sometime next year). He plans to do a capstone project on some aspect of this project.

### Activities and Findings

#### Research and Education Activities:

The submitted proposal contained three objectives, each with three or four null hypotheses:

1. Explore phylogenetic relationships within Maloideae s. l.
2. Study phylogeny of Rosoideae s.s., especially Potentilleae.
3. Bring all available data to bear on an overall phylogeny of Rosaceae.

In response to the budget reduction of about 40%, I eliminated objective 2, which was justified because (1) abundant evidence indicates that Rosoideae s.s. are monophyletic, which means that we can still consider an overall phylogeny of Rosaceae without sampling extensively within Rosoideae; (2) the interests and experience of the P.I. and the designated postdoctoral associate R. C. Evans are in Maloideae; and (3) our

collaborators (L. A. Alice and T. Eriksson) have a primary interest in Rosoideae s.s. and can take the lead in studying objective 2.

## Overall summary

This project concerns evolutionary relationships within the large and important rose family. This group contains numerous commercially valuable fruits - such as apples, pears, strawberries, cherries, peaches, plums, blackberries, and raspberries - and many horticulturally significant plants - such as roses, cotoneaster, lady's mantle, bridle-wreath, flowering quince, fire-thorn, cinquefoil, and others. Despite the role that these plants play in our daily lives and long scientific interest, we do not have a clear view of the evolutionary relationships of members of the family. A primary reason for this situation is that insufficient evidence has been accumulated to address the problem adequately. The chief accomplishment of this project has been to obtain DNA sequence data for a reliable chronicle of the evolutionary history of the rose family. These data are being compared with structural, chemical, and other data that have been accumulated for the family to create an overall picture of evolutionary relationships in the family. Such knowledge establishes a framework for studies of key groups within the family, provides insights into the evolution of interesting features in the family (such as the fruits), and contributes to our overall understanding of plant evolution.

## Findings:

The two major contributions of this project are better understanding of evolutionary relationships of the rose family and development of a low-copy-number nuclear gene (granule-bound starch synthase; GBSSI) for phylogenetic studies. Much of the results of this project is submitted and in preparation for submission for publication. Below are brief summaries of four of the most significant papers (see publications) coming from this project.

1. The granule-bound starch synthase (GBSSI) gene in Rosaceae: multiple putative loci and phylogenetic utility - This was the first phylogenetic study to use the 5' end of GBSSI. We sampled 1.8-2 kb, including two partial and seven complete exons and eight introns for 108 clones from 18 species in 14 genera representing all subfamilies of the Rosaceae and an outgroup (*Rhamnus*, *Rhamnaceae*). Phylogenetic analysis and southern hybridizations demonstrate two loci (GBSSI-1 and GBSSI-2) in all four subfamilies, even though only one locus had heretofore been documented from diploid flowering plants. Phylogenetic analysis further identifies four loci (GBSSI-1A, and GBSSI-1B, GBSSI-2A, and GBSSI-2B) in subfamily Maloideae, an allopolyploid group. Phylogenetic relationships inferred from GBSSI sequences are largely compatible with those from other genes. Large clades are marked by significant intron variation: a long first intron plus no sixth intron in Maloideae GBSSI-1, a long fourth intron in Rosoideae GBSS-1, and a GT to GC mutation in the 5'splice site of the fourth intron of all GBSSI-2 sequences.
2. The origin of the apple subfamily (Rosaceae: Maloideae) is clarified by DNA sequence data from duplicated GBSSI genes -- For 70 years the leading hypothesis for the origin of the Maloideae has involved wide hybridization between ancestors of two other subfamilies. The basis of this hypothesis is that Maloideae have a base chromosome number of 17, while other Rosaceae are mostly  $x=7, 8$  or  $9$ . To investigate this hypothesis we cloned and sequenced approximately 1.8 kb from the 5' portion of granule-bound starch synthase (GBSSI or waxy) genes for 70 clones from 23 Rosaceae genera. Previous studies demonstrated the presence of two copies in all Rosaceae (GBSSI-1 and GBSSI-2) and four in the Maloideae (GBSSI-1A, GBSSI-1B, GBSSI-2A, and GBSSI-2B). Parsimony and maximum likelihood analyses nest *Porteranthus* (*Gillenia*), a genus of the southeastern United States with a base chromosome number of 9, within either Maloideae GBSSI-1 or GBSSI-2 clades. Monophyly of Maloideae-*Porteranthus* clades is supported by bootstrap values approaching 100%, loss of the sixth intron in all GBSSI-1 sequences, intron alignability between genera, and numerous non-molecular characters. Our results falsify the wide-hybridization hypothesis and are consistent with a polyploid origin involving only members of a lineage that contained the ancestors of *Porteranthus*. Under this hypothesis, the subfamily originated in North America and the high Maloideae chromosome number arose via aneuploidy from  $x = 18$ .
3. A phylogeny of the Maloideae - Relationships of the approximately 30 genera of Maloideae have long been a challenge. Low levels of evolutionary divergence and intergeneric hybridization may have contributed to our poor understanding of the genera. Alignability of GBSSI introns, which is facile in the Maloideae, is not possible in any of the other major Rosaceae clades. Other data indicating little evolutionary divergence among genera of the Maloideae include the ease with which many different maloid genera may be graft successfully, numerous intergeneric natural hybrids, and low sequence divergence in five chloroplast regions (*matK*, *ndhF*, *rbcL*, *rps16* intron, and *trnT-L*). This lack of divergence is surprising in a group that extends back to at least the Middle Eocene. Past intergeneric hybridization could well have obscured the phylogeny with reticulation. The limited evolutionary divergence could be the result of a combination of relatively slow rates of evolution in woody plants or of continued gene flow between the genera. We have completed a data set of sequences for four chloroplast DNA regions -- *matK*, *ndhF*, *rps16* intron, and *trnT-L* - for a sample of 31 maloid genera. We have completed a data set of internal transcribed spacer (ITS) sequences, and have about 80% of a GBSSI data set for all four loci for the same sample of genera. Our goal is to provide a robust phylogeny of this subfamily and to provide insight into potential past episodes of hybridization.
4. A phylogeny of the Rosaceae - Along with collaborators, I am assembling a multi-gene data set to attempt to establish the overall phylogeny of the family. The largest Rosaceae phylogenetic study, the *rbcL* study of Morgan et al. (1994), did resolve numerous larger clades but did not resolve well the relationships among these clades. We will use several chloroplast regions, ITS, and GBSSI toward this effort. We plan to

present this phylogeny at the Rosaceae colloquium during the American Society of Plant Systematists (ASPT) meetings in Madison Wisconsin in August 2002 (see below). We are in the process of determining who among about six collaborators will be responsible for which genes and other components of this large undertaking.

#### **Training and Development:**

1. The postdoc, Dr. Rodger C. Evans, obtained a lot of new data, especially using granule-bound starch synthase (GBSSI) sequences and considerable experience in cloning and sequencing a low-copy-number nuclear gene. He coauthored one significant publication, submitted another, and has several others in preparation. He developed a number of collaborations and was selected for a tenure-track position at Acadia University in Wolfville, NS.
2. Dr. Timothy A. Dickinson's visit to the Campbell lab at the University of Maine provided him with an opportunity to learn techniques of molecular systematics from C. S. Campbell, post-doc R. C. Evans, and others at the University of Maine. Funding for this visit came from Dickinson's home institution, the Royal Ontario Museum (ROM) and from his research grant from the Natural Sciences and Engineering Research Council of Canada (NSERC). Supplementary funding to help offset living expenses in Orono was obtained from the University of Maine. Using DNA extracted from samples collected prior to and during the sabbatical, substantial progress was made toward an ITS phylogeny of *Crataegus*, with *Mespilus* and other, apparently closely related Maloideae (e.g. *Amelanchier*) as outgroups. Amplification and sequencing of the chloroplast gene *trnL* was also done, in order to evaluate its utility in *Crataegus* and to collect data for a collaborative multi-gene study of Maloideae phylogeny. The ITS results were presented at Botany 2000 in Portland OR in August 2000. These results indicate that at least two variants of ITS are present in *Crataegus*, of which only one (apparently restricted to *Crataegus*) shows phylogenetically significant variation. Further work has been delayed until recently by the move of the ROM's botanical collections from offsite locations into the main Building of the ROM. Dickinson's work in Maine was facilitated by access not only to the resources in Campbell's lab and those of the Department of Biological Sciences, but also to the University of Maine DNA Sequencing Facility ([www.ume.maine.edu/dnaseq/](http://www.ume.maine.edu/dnaseq/)).
3. Jenny Smedmark wanted GBSSI sequences to provide perspective on the history of extensive polyploidy in *Geum* (there are few known diploids). This gene has worked marvelously in this regard, as Smedmark was able to get most of the expected number of GBSSI copies in tetraploids and hexaploids. She spent a month in our lab cloning GBSSI for her sample. She clearly demonstrates that the polyploids are allopolyploid and has identified one parental genome in several cases.

#### **Outreach Activities:**

None

#### **Journal Publications**

Evans, R.C., L.A. Alice, C. S. Campbell, E. A. Kellogg, and T.A. Dickinson., "The granule-bound starch synthase (GBSSI) gene in Rosaceae: multiple putative loci and phylogenetic utility.", *Molecular Phylogenetics and Evolution*, p. 388, vol. 17, (2000). Published

Evans, R.C. and C.S. Campbell., " The origin of the apple subfamily (Rosaceae: Maloideae) is clarified by DNA sequence data from duplicated GBSSI genes.", *American Journal of Botany*, p. , vol. , ( ). Submitted

Campbell, C.S., R.C. Evans, and others., "A phylogeny of the Maloideae", *Systematic Botany*, p. , vol. , ( ). in preparation

Campbell, C.S., R.C. Evans, and others., "A phylogeny of the Rosaceae", *American Journal of Botany*, p. , vol. , ( ). in preparation

#### **Books or Other One-time Publications**

#### **Web/Internet Site**

#### **Other Specific Products**

#### **Contributions**

#### **Contributions within Discipline:**

The major contributions within the discipline are better understanding of evolutionary relationships of the rose family and development of a low-copy-number nuclear gene (granule-bound starch synthase; GBSSI) for phylogenetic studies. We are the first to use the 5' end of GBSSI in

phylogenetic studies. More information about the evolutionary relationships within the Rosaceae is presented in the section on activities and findings.

A major contribution of this project is the organization of a colloquium, entitled 'Rosaceae phylogeny: Current knowledge, problems, and prospects.' This colloquium has been approved by the ASPT. The primary objective is to encourage synthesis and discussion of recent molecular and other data relevant to Rosaceae phylogeny. The conceptual focus is that a phylogenetic perspective will advance our understanding of evolution of the Rosaceae, which has been hampered by its large size, considerable geologic age, indistinct boundaries between taxa, hybridization, polyploidy, agamospermy, and complex relationships among the taxa. The colloquium organizers are Christopher S. Campbell and Timothy A. Dickinson.

Pooling large amounts of recently collected data from a large number of genes and other sources and sharing perspectives will greatly enhance our understanding of Rosaceae phylogeny. This colloquium should stimulate and direct future research in this large, ecologically and economically important clade. After an introduction and overview of the major challenges of the topic, we would consider the impact of hybridization, polyploidy, and agamospermy on Rosaceae phylogeny, demonstrate the phylogenetic utility of reproductive structures, and focus on important and challenging groups within the family. We would conclude the colloquium with a phylogeny of the family based on all available data. The presenters were chosen for their work on Rosaceae phylogeny and ability to contribute to a balanced presentation. Ongoing collaborations and communications among the presenters provide a network for integration of different perspectives. We have a commitment from the presenter for all presentations except numbers 3 and 8, and we expect commitments for these two presentations.

Below is a tentative list of: titles of presentations and names of presenters

1. Rosaceae phylogeny: An introduction and overview - T. A. Dickinson
2. Hybridization, polyploidy, and phylogeny in the Rosaceae - J. Smedmark
3. Agamospermy and evolution in the Rosaceae - B. Eriksen
4. Floral development and Rosaceae phylogeny - R. C. Evans
5. Reproductive character evolution in Maloideae - D. R. Morgan and K. R. Robertson
6. Evolution and phylogeny of Rosoideae - T. Eriksson
7. Phylogeny and relationships of prunoids of the Rosaceae - D. Potter
8. Phylogenetic insights into a taxonomically challenging group (Rubus) - L. A. Alice
9. A phylogeny of the Rosaceae - C. S. Campbell

Much of the results of this project is published, submitted for publication, or in preparation for submission for publication.

**Contributions to Other Disciplines:**

None

**Contributions to Human Resource Development:**

Our contributions to human resource development are in the area of training, detailed in another section of this report.

**Contributions to Resources for Research and Education:**

None

**Contributions Beyond Science and Engineering:**

None.

**Categories for which nothing is reported:**

Organizational Partners

Any Book

Any Web/Internet Site

Any Product